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(54) Title: **PROCESS FOR PRESERVING FOODSTUFF**

(57) Abstract: A process for preserving foodstuff, such as fruits, berries or vegetables, wherein the foodstuff is pre-treated in liquid medium so as to displace gases present in the foodstuff, and the pre-treated foodstuff is subjected to a pressure of at least 300 MPa in order to inactivate enzymes and micro-organisms and parts thereof. According to the invention the foodstuff is contacted in the liquid medium with calcium ions and pectinmethylesterase at reduced pressure. By infusing the enzyme and calcium into the tissue of the foodstuff it is possible to improve the firmness of the foodstuff while simultaneously deaerating the product.

Process for preserving foodstuff

The present invention concerns a process according to the preamble of claim 1 for
5 preserving foodstuff, such as fruits, berries and vegetables, as whole or in pieces, by
treating them under high pressure.

According to a process of the present kind, the foodstuff is deaerated and subjected to a
pressure of at least 200 MPa in order to inactivate enzymes and/or micro-organisms and
10 parts thereof.

The growing consumer demand for high quality, minimally processed, additive-free and
microbiologically safe foods, has created a need for new food processing methods. The
high-pressure technology has proven to be a potential new method allowing the production
15 of foods, which fulfil the expectations of the consumers.

The aim of the pressure treatment is to inactivate micro-organisms and enzymes natively
contained in the foodstuff which could cause deterioration of the product during storage.
By pressure treatment it is, thus, possible to increase the shelf life of the product. The
20 benefit of using high pressure instead of heat for preservation is that the aroma, colour and
nutritional value of the fresh product is better maintained, while the pressure treatment can
be carried out at a reduced temperature.

The preservation of porous fruits and vegetables is often problematic, since they contain
25 significant air voids within the tissue. During pressure-processing the air is not removed
from the product. On being subjected to pressure any gases contained in a porous material
will be almost completely compressed, which can cause an irreversible collapse of the
tissue. The oxygen dissolved in the product causes trouble also in keeping quality. Various
oxidative reactions, by which the quality of the product deteriorates, can take place in the
30 product during storage. These reactions can be both non-enzymatic and enzymatic,
depending on how well the enzymes have been deactivated. Reactions affecting product
quality are for example discoloration (e.g. browning), formation of off-flavours and
decomposition of vitamin C.

Foodstuff can be deaerated by vacuum packaging, i.e. by placing the products in an air-tight package, which is then evacuated to remove gases in the pores of the foodstuff. This kind of packaging will, however, damage the texture of many soft and porous berries and fruits.

5

It is known in the art to produce a fruit-containing jelly which is claimed to hold the flavour of the raw fruit and having improved preservability for long times by mixing raw fruit, a syrup liquid and a gelling agent in a flexible container which then is subjected to a pressurising treatment (JP Patent Application No. 19900271689). This pre-treatment in
10 liquid medium is carried out at a pressure of < 750 mmHg.

Similarly to vacuum packaging, soaking of fruits, berries and vegetables in a liquid medium as well as high pressure treating can cause a considerable softening of the treated tissue.

15

It is an aim of the present invention to eliminate the problems of the prior art and to provide a process for successfully preserving porous fruits and vegetables in liquid media, while maintaining the aroma, colour, nutritional value and texture of the fresh raw material as well as possible.

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The present invention is based on the concept of treating the foodstuff in a liquid medium containing the enzyme pectinmethylesterase (in the following also abbreviated "PME") and calcium ions under vacuum prior to high pressure processing. In connection with the invention it has been found that by infusing the enzyme and calcium into the tissue of the
25 fruit or vegetable it is possible to improve the firmness of the foodstuff while simultaneously deaerating the product. Gases are, viz., drawn out from the fruit or vegetable while it is submerged in the enzyme solution under vacuum and infusion occurs when the vacuum is released.

30 More specifically, the present invention is mainly characterised by what is stated in the characterising part of claim 1.

The present invention provides considerable advantages. Thus, the firmness of the berries pre-treated by a liquid containing calcium ions and PME is approximately ten times higher

than that of berries that are not pre-treated at all and five times higher than that of berries pre-treated in only water. Surprisingly, it has been found that the pressure resistance of PME is good and the enzyme is probably not completely deactivated during high pressure treatment. On the contrary, it appears that high-pressure treatment may even increase the
5 activity of PME.

By replacing the gas in the voids with water, less tissue damage can be expected due to pressurisation, since the compressibility of water is much more limited than that of gases, about 12 % at a pressure of 400 MPa. The removal of air from the product being pressure
10 treated is also beneficial for the shelf-life of the pressurised product. However, it has been found with, e.g. strawberries, that soaking in only water before pressure treatment is not enough for maintaining the shape and firmness of the fresh berry. Thus, the present enzyme treatment is necessary.

15 According to the present invention, the foodstuff which is subjected to the present processing is at least partially derived from plants and it comprises cell walls containing lignocellulosic material. Preferably, the foodstuff is selected from berries, fruits or vegetables, in particular fruits, vegetables and berries having soft cell walls and, thus, having a sensitive texture, that may be damaged under the influence of high pressure
20 treatment.

The berries treated typically have a solid matter or dry matter (these expressions are herein used interchangeably) content of 15 % or less, such as about 8 to 12 %. These kinds of berries are, for example, the following: strawberries, raspberries, blueberries, cranberries,
25 cloudberry, lingonberries, red whortleberries, arctic bramble, black, red and white currants, and crowberries. Suitable fruits are exemplified by cherries, plums, apricots, peaches, apples, avocado, date and citrus fruits. Examples of vegetables include tomatoes, potatoes, cucumber, cabbage, squash, pickles, pumpkin, pepper, beans, peas, lenses, garlic, onion, olives and mushrooms.

30

In the following, the present process will be described with particular reference to the processing of fresh strawberries, but it should be noted that the same procedures can be followed for processing other berries, fruits and vegetables – fresh as well as prepared. In

addition to treating whole berries, it is of course also possible to use berry pieces, such as slices or cubes.

Figure 1 shows the relative firmness of high pressure treated strawberry pieces of Example 1 by means of a bar chart. The letters at the top of each column show the result of the statistical analysis. The means of each sample are compared pairwise and if the means show similar letters there is no statistically significant difference between the means.

The processing according to the present invention comprises two main steps: pretreatment and high pressure processing.

Before treatment, the fresh fruits and vegetables are cleaned and optionally peeled, cut and washed. The berries are typically dehulled and optionally cut into pieces. After this preliminary step, the foodstuff is contacted with calcium ions and an enzyme having pectinase activity, preferably pectinmethylesterase, in an aqueous medium. The contacting is carried out in a solution for a predetermined period of time. In order to promote the impregnation of the berries with the calcium ions and the enzyme, reduced pressure is applied. Preferably, the present vacuum impregnation treatment is carried out by immersing the pore structure of the fruit into a liquid. Simultaneously, reduced pressure is imposed on the solid-liquid system, and after the predetermined time, the system is brought back to atmospheric pressure. During the vacuum step the internal gas in the product pores expands and partially flows out.

The aqueous solution used for the vacuum step (treatment at reduced pressure) comprises a dissociating calcium compound which will provide calcium ions in a liquid medium. Both inorganic or organic calcium salts and compounds, e.g. calcium chloride, calcium hydroxide, calcium ascorbate and calcium gluconate, can be used. The concentration of the calcium ions in the solution or dispersion is about 0.001 - 0.05 mol/l, preferably 0.01 - 0.2, in particular 0.07 - 0.15 mol/l, whereas the concentration of the inorganic salt, such as calcium chloride, is about 0.5 to 2 wt-%, preferably about 0.8 to 1.2 wt-%, calculated from the weight of the medium. The amounts of the organic salts can be, calculated by weight, somewhat greater. Too large amounts may impair gelling of the pectin during a subsequent food processing step, such as jam preparation, causing the medium to become fluid. High amounts of calcium ions may also affect the taste of the foodstuff.

- Preferably, the berries are simultaneously treated with both the dissociating calcium compound and an enzyme. Therefore the above solution or dispersion also contains an enzyme capable of achieving hydrolysis of the methyl ester bonds present in the pectin of the berry. Pectin is a high molecular weight heteropolysaccharide, which consists of a chain structure of α -(1,4)-linked D-galacturonic acid units containing blocks of rhamnose-rich regions. Other constituent sugars are attached in side chains, the most common being D-galactose, L-arabinose and D-xylose. The carboxyl groups of the galacturonic acid are partly esterified by methyl groups. Some of the hydroxyl groups on C₂ and C₃ may be acetylated. The pectinase used primarily catalyzes the cleavage of the ester bonds between the methyl groups and the carboxyl groups thus forming free acid groups. The free acid groups react with divalent cations and calcium pectate is thus formed, which is assumed to anchor the pectic substances and result in an overall increase in firmness.
- The pectinase used is preferably a pectinmethylesterase (EC 3.1.1.11). There are commercial products available (supplied by Novo Nordisk, Sigma and Röhm & Haas), some of which, depending on the cultivation conditions, exhibit side activities of other pectinases, proteases and cellulases. These side activities may somewhat impair the action of the PME on the firmness of the berries. The enzyme dosage is generally in the range of 10 to 200,000 nkat/kg, suitably 10 to 100,000 nkat/kg, preferably about 15 to 10,000 nkat/kg, in particular about 20 to 6000 nkat/kg, calculated from the fresh weight of the substrate, e.g. the berries subjected to the pretreatment. The enzyme activity can be calculated as explained in more detail in connection with the example.
- It should be noted that it is also possible to treat the berries with calcium ions and pectin methyl esterase or pectin methyl esterase and calcium ions, respectively, in successive treatment steps. For that purpose two different treatment liquids can be prepared, one containing the source of calcium ions and one containing the enzyme. The pressure used for such successive treatment steps can be the same or different as explained below.
- In addition to the calcium compound and the enzyme (or either) the pretreatment medium may contain other components such as buffering agents and other agents for adjusting the pH. The medium has generally a pH of about 5 to 7.

The treatment time of the vacuum step is usually 1 to 60 minutes, depending on the foodstuff treated and the magnitude of the reduced pressure. The pressure is usually between 0.01 to 100 kPa, in particular about 1 to 40 kPa. For strawberries, a treatment time of 10 min is enough at a pressure of 13 to 40 kPa, (about 100 to 300 mmHg), whereas
5 shorter treatment times can be used at lower pressures. The longer the treatment, the more gentle vacuum step can be used. If the pressure is too low, the berries will be damaged. For berries and fruits having a hard skin, longer treatment periods and lower pressures can be used. The temperature of the vacuum step is about 5 to 60 °C, preferably 30 to 50 °C. The upper limit is determined by the temperature acceptable for the foodstuff, because the
10 enzyme usually stands temperatures of up to about 60 °C.

The pretreatment is carried out in order to stabilize the fibrous structure of the foodstuff. With strawberries, the vascular tissue, the cortex and the pith of the berries will be stabilized. As a result of the treatment, the berries will contain calcium ions in an amount
15 of about at least 200 mg/kg d.m., preferably 230 to 1000 mg/kg. It has been found that the combination of pectinmethylesterase with calcium ions will strongly increase the amount of calcium bound to the berries.

During the second main step, the foodstuff is subjected to high pressure treatment in the
20 presence of a liquid medium. After the pre-treatment, the berries, fruits or vegetables are preferably drained for removing surplus liquid and placed into plastic pouches or other flexible packages suitable for high pressure processing. The pouches can be manufactured from a number of suitable materials in particular including multilayer plastic films and aluminium based plastic films. As regards suitable materials, reference is made to the
25 article by B. Mertens [Developments in High Pressure Food Processing (Part 2), International Food Manufacturing ZFL 44 (1993) 4, 182 – 187]. The packages are then filled with a liquid, such as water, juice or sugar liquor or brine, and heat sealed with minimal headspace. Alternatively or additionally, various gelling agents and/or thickening agents can be used. Examples of such gelling or thickening agents include pectin, starch,
30 cellulose derivatives, carragenan, agar, gelatine, alginates, gellan gum and guar gum.

It is also possible to subject the foodstuff to high pressure treatment soaked in the pre-treatment liquid medium. In that case, the foodstuff need not be drained at all or is only partially drained after the pre-treatment. Sugar, salt or other agents including gelling and/or

thickening agents, optionally in liquid medium, are then added to the pre-treatment medium. These agents may also be added before the actual pre-treatment.

The product placed in a liquid medium and optionally packed in a flexible container, as described above, is then transferred to a high-pressure treatment apparatus. The foodstuff is placed in a high pressure vessel which typically is filled with a liquid pressure medium comprising, e.g. water, oil, glycol, hexane or a mixture thereof. The pressure during the treatment is higher than 200 MPa, preferably it is at least 300, in particular about 400 to about 700 MPa, The duration of the treatment is 1 – 60 min. By using continuously operated high pressure equipment the treatment time can be further cut and the present invention also covers pressure treatment carried out by means of short pressure shocks having a duration of some seconds. The temperature during pressure processing is kept at 0 – 60 °C, preferably 0 – 40 °C by means of a temperature controller unit. The higher the pressure and temperature and the longer the treatment time, the better inactivation of micro-organisms and enzymes can generally be expected, while the opposite is usually desirable when the maintenance of the original texture is the aim. The suitable pressure, temperature and time combination depends strongly on the product treated.

For industrial applications, any high pressure equipment may be employed for generating the required high isostatic pressure. Typically, the equipment comprises low alloy steel vessels having a cylindrical configuration. The pressure is generated through direct or indirect compression of the pressure medium. The direct compression is obtained by using a piston-type press. The indirect compression can be attained by using a high pressure-intensifier pump. For further details on high pressure equipment, reference is made to the article by B. Mertens [Developments in High Pressure Food Processing (Part 1), Internation Food Manufacturing ZFL 44 (1993) 3, 100 – 104].

Thus, in summary, the preferred embodiment of the present process, applied to strawberries and similar berries having a sensitive and soft texture, comprises the following steps:

- immersing selected berries into an aqueous solution containing a calcium salt and pectinmethylesterase,
- maintaining said solution at reduced pressure for a predetermined time,
- draining the treated berries to separate at least a part of the aqueous solution,

- optionally adding an aqueous solution containing carbohydrates, such as sugar, and
- subjecting the berries to a pressure of 400 to 700 MPa.

The example below illustrate in more detail the benefits of the present invention.

5

Example

Materials

- 10 Fresh of Belgian origin were used for pressure treatment. A liquid preparation of a microbial pectinmethylesterase from *Aspergillus oryzae* (E.C. 3.1.1.11, Novo Shape, Novo Nordisk Ferment Ltd., Dittingen, Switzerland) was used as enzyme. The enzyme hydrolyses the methyl esterified galacturonic acid units of pectic substances. The product used was free of polygalacturonase activity. The PME activity was assayed by titration of
- 15 the liberated carboxyl groups of 1-% citrus pectin with 50 mM NaOH solution using an automatic titration at room temperature. One unit of PME activity (nkat) was defined as the amount of enzyme that liberated 1 nmol of carboxyl groups per second under the assay conditions. The PME activity was 100 000 nkat/ml.

20 Pretreatment

- The fresh strawberries were cut into four pieces in the longitudinal direction. 60 g of berry pieces were soaked in water (72 ml) or in water containing pectinmethylesterase and/or calcium ions as shown in Table 1. A non-pretreated sample was used as reference. The concentration of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was 1 % (w/w). The enzyme dosage was 200 μl /100 g fresh
- 25 strawberries. The soaking was carried out in a vacuum chamber (W.C. Heraeus GmbH, Germany) at a reduced pressure of 13.3 kPa (100 Torr) for 10 min. A light weight was put on the strawberries to ensure that they were completely immersed in the liquid. After soaking the berries were drained in a strainer and the berry pieces were placed into flexible plastic (PA/PE) pouches. The pouches were then filled with 10 % sugar solution and heat
- 30 sealed.

Table 1. List of pretreatments.

Sample no.	Pretreatment at reduced pressure		
	Water	PME	Ca
0	No pretreatment		
1	x	-	-
2	x	x	-
3	x	-	x
4	x	x	x

High pressure treatment

- 5 The pressure treatments were performed in a multivessel high-pressure device (HPIU-10000-AT, Resato International, The Netherlands). The maximum working pressure of the unit is 1000 MPa. The six vessels of the unit have an internal diameter of 25 mm and a capacity of about 60 cm³. The vessels are surrounded by a water jacket, through which water from a thermostatically controlled water bath is circulated. Pressure is created by
- 10 means of a pressure intensifier. The pressure-transmitting medium is glycol.

- The samples were pressurized at a rate of about 50 MPa/min up to 500 MPa. The time course of the experiment began when the desired pressure was reached. The holding time was 15 min. The vessels were thermostatically maintained at 25°C, but a slight increase in
- 15 temperature was observed during pressurization. This temperature increase was approximately 10°C.

Firmness measurements

- For the measurement of the firmness of the berries a Texture Analyser (TA-HDi, Stable
- 20 Micro Systems, England) fitted with a 5 kg load cell was used. The berry pieces were sheared and compressed in the lateral direction with a knife blade (HDP/BSK) at a speed of 2 mm/s. The starting position of the blade was 20 mm above the base plate and the compression distance 19.5 mm. The shearing force was plotted as function of time and the firmness was defined as the area under the measured curve. At least ten strawberry pieces

of each sample were measured. The means of the samples were compared by a multiple range test procedure (Fisher's least significant difference) using STATGRAPHICS® *Plus* (Version 3.1, Statistical Graphics Corp., 1994-1997). With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

5

Results

The results of the firmness measurements of the high pressure treated strawberries are presented in Figure 1. The firmness values were converted to relative values, with the non-pretreated reference sample having a firmness of 1. A pretreatment in only water did not significantly increase the firmness of the berries. A slight increase was observed for the berries soaked in a liquid containing either enzyme or calcium ions. By far the best result was, however, obtained when both enzyme and calcium ions were present in the soaking liquid, giving a firmness that was about nine times higher than that for the berries that were not pretreated at all.

15

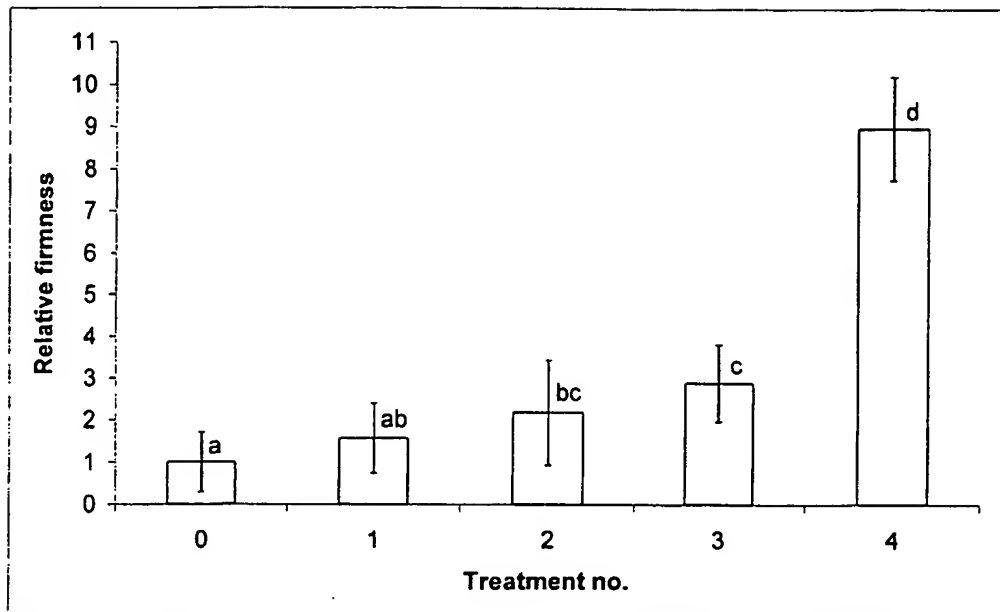
Claims:

1. A process for preserving foodstuff, wherein
 - the foodstuff is pre-treated in liquid medium so as to displace gases present in the foodstuff, and
 - the pre-treated foodstuff is subjected to a pressure of at least 300 MPa,characterized by
 - contacting the foodstuff at reduced pressure in the liquid medium with calcium ions and pectinmethylesterase.
2. The process according to claim 1, wherein the liquid medium comprises an aqueous solution containing about 0.001 – 0.5 mole/l, preferably 0.01 – 0.2, in particular 0.07 – 0.15 mole/l, calcium ions and about 10 to 200,000 nkat/kg pectinmethylesterase.
3. The process according to claim 1 or 2, wherein the foodstuff is pre-treated in aqueous medium with 0.5 to 2 wt-% of a dissociating calcium compound and 20 to 6000 nkat/kg pectinmethylesterase.
4. The process according to any of claims 1 to 3, wherein the pre-treated foodstuff is placed into plastic pouches or similar flexible packages suitable for high pressure processing.
5. The process according to claim 4, wherein the packages are filled with a liquid, and they are heat sealed with minimal headspace.
6. The process according to claim 5, wherein the packages are filled with water, fruit juice, sugar liquor or brine.
7. The process according to any of claims 4 to 6, wherein the packages are filled with a gelling agent and heat sealed.
8. The process according to any of claims 4 to 7, wherein the liquid medium used for pre-treatment of the foodstuff is partially or *in toto* drained from the pre-treated foodstuff.

9. The process according to any of claims 1 to 5, wherein the foodstuff is subjected to a pressure of at least 300 MPa in the presence of the liquid medium used for pre-treatment.
10. The process according to any of claims 1 to 9, wherein the foodstuff is treated in a high pressure vessel at a pressure of 400-700 MPa for 1 - 60 min.
11. The process according to any of claims 1 to 10, wherein the temperature during the pressure processing is kept at 0-40 °C.
12. The process according to any of the preceding claims, wherein the foodstuff is selected from fruits, vegetables and berries having soft cell walls.
13. The process according to claim 12, wherein the foodstuff comprises berries having a solid matter content of 15 % or less.
14. The process according to claim 13, wherein the berries are selected from the group of strawberries, raspberries, blueberries, cranberries, cloudberrries, lingonberry, red whortleberry, currants, arctic bramble, and crowberry.
15. The process according to claim 12, wherein the fruits are selected from cherries, plums, apricots, peaches, apples, tomatoes, avocado, dates and citrus fruits.
16. The process according to claim 12, wherein the vegetables are selected from tomatoes, potatoes, pickles, pumpkins beans, peas, lenses, garlic, onion, olives and mushrooms.
17. The process according to any of claims 1 to 16, wherein the pre-treatment is carried out at a temperature of 20 to 60 °C.
18. The process according to any of claims 1 to 17, wherein the pre-treatment is carried out at a pressure of 0.01 to 100 kPa, preferably 1 to 40 kPa.
19. The process according to any of the preceding claims, wherein berries are treated by – immersing selected berries into an aqueous solution containing a calcium salt and pectinmethylesterase,

- maintaining said solution at reduced pressure for a predetermined time,
 - draining the treated berries to separate at least a part of the aqueous solution, and
 - subjecting the berries to a pressure of 400 to 700 MPa.
- 5 20. The process according to any of the preceding claims, wherein the calcium compound is selected from the group of calcium chloride, calcium hydroxide, calcium ascorbate or calcium gluconate.

1/1

*Fig. 1*

INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A23L 3/015, A23B 7/155

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A23B, A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	Patent Abstracts of Japan, abstract of JP 42-18346 A (TOPPAN PRINTING CO LTD), 7 August 1992 (07.08.92) --	1-20
A	WO 9852423 A3 (GIST-BROCADES B.V.), 26 November 1998 (26.11.98), page 4, line 16 - line 26, claims 1-10, abstract --	1-20

☒ Further documents are listed in the continuation of Box C.
 ☒ See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 01/00127

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT
Information on patent family members

28/05/01

International application No.

PCT/FI 01/00127

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